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ORIGINAL ARTICLE

Identification and Isolation of fungus from food and determination of its antibacterial activity

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ABSTRACT

The issue of drug resistant pathogens is the most well-known issue now days. Irresistible and parasitic infections are still the second driving reason for death. This is the major cause to examine their antibacterial activity. The antimicrobial compounds produced by fungus naturally are the most vital source of their discovery. Parasites are easy and valuable source with the great pharmaceutical potential. This study was led to explore that whether the infectious metabolites have ability to repress or execute the human pathogenic microscopic organisms. The parasitic metabolites were segregated from *Aspergillus niger* , *Aspergillus fumigatus* , *Aspergillus flavus* , *Rhizopus* and *Penicillium* and check their antibacterial action against human pathogenic microorganisms, for example, *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeroginosa*. Zone of inhibition were measured by agar well diffusion method and pharmacological activity analyzed by qualitative test, for example, glycoside, alkaloid and tannins test. The results demonstrated that just Aspergillus fumigatus have pharmacological activity and it give zone of restraint against human pathogenic microbes. Other fungi for example, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* and *Penicillium* did not give any zone of inhibition against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeroginosa*. *Aspergillus fumigatus* metabolites can be use for the preparation of antibiotic.

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Introduction

Today, the major problem is the dominance of infectious disease causing a vast damage to the population. According to the WHO (World Health Organization) in 2016 the great influence of diseases was reported, dominant microbial flora and parasitic diseases were reported as the main reason of death, constituent about 18.4% all over the world (1). This causes the need to evaluate the antimicrobial drugs against different pathogens (2).

As of late there have been an expanding number of publications with respect to assessment of growths delivering antimicrobial substances (3). Growths are equipped for delivering differing class of common mixes as optional metabolites. In fact, a percentage of the metabolites have helpful biochemical exercises, for example, phytotoxicity, cytotoxicity, nematocidal and antimicrobial properties (4). In the most recent era, the study enlighten that organisms are significant source of strong bioactive secondary metabolites. Transmissible metabolites can be utilized as a part of important technique, for instance, therapeutic uses, it has been assessed that more than 40 % of prescriptions utilized today have their starting point as a part of normal items among which are parasitic metabolites (5).

Secondary metabolites are synthetic elements discovered fundamentally in plants, organisms, and microorganisms. Secondary metabolites contain particles, for example, hormones, anti-toxins, and poisons and give unlimited

wellsprings of pharmaceuticals. Be that as it may, interest for these mixes is impressive items are injurious (e.g., mycotoxins), while others are useful (e.g., anti-microbials) to mankind (6).

Aspergillus, a variety included filamentous growths, is a broadly considered gathering of sac organisms containing a various cluster of both valuable and pathogenic species. Like all organisms, *Aspergillus* species are eukaryotic and have a cell divider made of chitin. (7).

A characterizing normal for "*Aspergillus*" is their capacity to create secondary metabolites in response to ecological parameters, permitting them to adjust to mind complex and evolving situations. These secondary metabolites permit growths to either build their own wellness or abatement an encompassing life form's wellness, guaranteeing survival and generation. A couple of the numerous metabolites blended by *Aspergillus* that have been found hold awesome significance in human application; a couple of illustrations incorporate the cholesterol decreasing medication lovastin, the anti-infection penicillin, and the pathogenic human poison aflatoxin (7).

As specified over, the utilization of "*Aspergillus*" secondary metabolites can be found in a few human therapeutic applications, for example, the anti-toxin penicillin (7). Metabolites are of extraordinary enthusiasm well beyond the microbial domain, incorporating significance in therapeutic, mechanical, and farming fields. For instance, it has been demonstrated that the larger part of hostile to cancer-causing and tumor stifling atoms used in current medication have beginning in normal metabolites (8).

In 1960s Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified as a nosocomial pathogen (9), MRSA disease can cause the late hospitalization or surgery. Intensive care Units (ICU), dialysis and indwelling percutaneous medicinal instruments and catheters are the main reservoirs of these pathogens.

Pseudomonas aeruginosa keeps on being a noteworthy pathogen among patients with immunosuppressant, cystic fibrosis, harm and injury. *Pseudomonas* aeruginosa causes everlasting respiratory illness, and the elastase compound that it produces expands the penetrability of aviation route epithelial cells inferable from the interruption of tight intersections. *P. aeruginosa* is additionally embroiled in delayed endless rhino sinusitis (10).

The study is to see if the parasitic metabolites have the ability to repress bacterial pathogens. Human pathogens that bring about sustenance conceived contaminations or nourishment harming can be counteracted by the utilization of these anti-microbials or their changed structures either as nourishment additives or as an ordinary anti-microbial to cure Urinary tract disease and throat disease and this study is likewise composed that whether parasites can restrain the development of pathogenic miniaturized scale life forms and this work additionally show an fascinating base up way to deal with the revelation of new antimicrobial mixes.

Materials and Method

Bacterial culture: *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeroginosa* were collected from the clinical laboratory.

Fungal cultures: Fungus was isolated by different spoiled food and vegetables using Czepekdox agar medium and identified the colonies by scotch tape method.

Preparation of fungal extracts: The fungal colonies were inoculated in Czepekdox broth and incubated for 5 days. Metabolites and growth produced were filtered by filter paper and then centrifuged at 3000 rpm and separate the supernatant for further use.

Qualitative Analysis Of Metabolites Produced By Fungi: The fungal extracts were subjected to qualitative analysis for the identification of compounds with the help of following test.

Test for Alkaloids: Few drops of Mayer's reagent were introduced in fungal extract. Positive test gave the yellowish precipitates

Test for glycosides: 0.5 ml acetyl chloride and few drops of ferric chloride and Conc. Sulphuric acid were added in the extract with acetic acid. Positive test gives the bluish

green color in the upper layer and reddish brown color at the junction of two layers.

Test for tannins: add 1 ml of water and 1-2 drops of ferric chloride to 0.5 ml of extract. positive results give the blue color for Gallic Tannis and green black color for Catholic Tannis.

Screening of the fungal extracts for anti-microbial activity: the lawns of *Pseudomonas* aeruginosa, *Staphylococcus* and Streptocus were made on Mueller Hinton Agar. The wells were punched and filled with 50 µl fungal extract. incubated at 37°C for 24 hours.

Results and Discussion

The study was carried out to study the fungal metabolites having capability to inhibit or eradicate pathogenic bacteria. Human pathogenic bacteria that are able to cause diseases in humans can be prevented by the use of these antibiotics and this work also demonstrates an interesting bottom-up approach to the discovery of new antimicrobial compounds. Different clinical sample were collected from different laboratories of Karachi. The Urine, skin and throat sample were gathered to confine pseudomonas, Staphylococci and Streptococci. Gram staining was done to confirm the purity of culture. After that it was confirmed by confirmatory test, *Staphylococcus aureus* were streak on nutrient agar plate then number of colonies were isolated, it was then streak on mannitol salt agar for their confirmation S. *aureus* appear as yellow colonies with yellow zones in the media. Then it was further confirm by catalase, coagulase and oxidase test it showed catalase positive, coagulase positive and oxidase negative. Colonies of *Streptococcus pyogenes* cultivated on blood agar S. *pyogenes* produces big zones of beta hemolysis on blood agar plate. S. *pyogenes* showed oxidase negative, catalase negative and coagulase positive. *Pseudomonas aeroginosa* were cultured on Tryptic soy agar it smell is describe as grape like on tryptic soy agar and shape of colonies were circular. *Pseudomonas aeroginosa* showed catalase positive, oxidase positive and coagulase negative.

Fungus were isolated from spoiled food (tomato, cauliflower, lemon, carrot and banana) from different areas of Karachi into sterile polythene bags and brought to laboratory for processing. Czepekdox agar medium was used for the growth of Fungus. Fungal colonies were then identified by staining them with lacto phenol cotton blue. And based on microscopic observation and lacto phenol blue staining the isolated organisms were found to be *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* and *Penicillium*.

In glycoside test the results were observed that *Aspergillus fumigatus* demonstrated reddish brown color at the intersection of two layer and bluish green show up in the upper layer. *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* and *Penicillium* did not form reddish brown colour at the intersection of two layer and somewhat blue green shading show up in the upper layer.

In alkaloid test, turbidity and yellowish precipitate was seen in *Aspergillus fumigatus* extract, which show that *Aspergillus fumigatus* have an extensive variety of pharmacological exercises including hostile to malarial (e.g. quinine), antiasthma, hostile to harmful exercises. Furthermore, no turbidity and yellowish hasten were found in *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* and *Penicillium* it demonstrate that these microorganisms might not have pharmaceutical activities since they are impartial compound which don't respond with any corrosive or base.

In tannins test blue color for Gallic tannins or green dark for catholic tannins was not found in *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* and *Penicillium*. It show that these creatures does not have pharmacological activities (inhibition of carcinogenesis, host-mediated antitumor activity, antiviral activity. Subsequently the compounds are recognized to be neutral compounds which don't respond with any corrosive or base

Figure 1. Alkaloid test

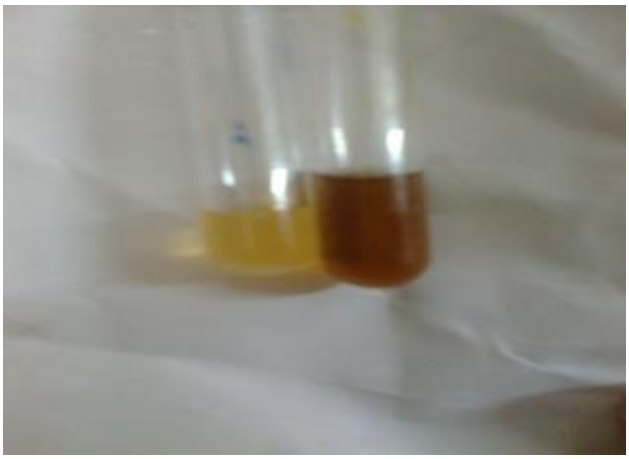

Figure 2. Tannins test

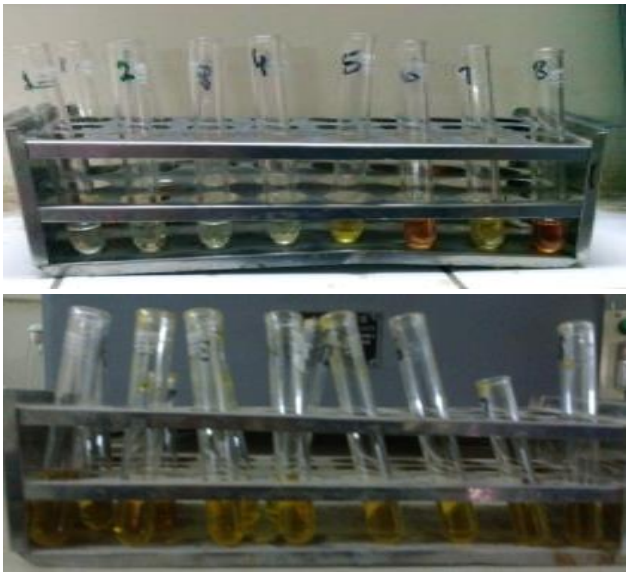

Figure 3. *Aspergillus flavus* , *Aspergillus niger* , *Rhizopus* and *Penicillium* showing negative result of Alkaloid and Tannins test.

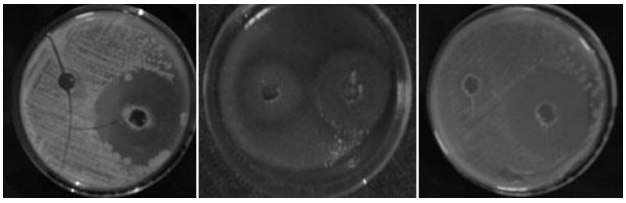

Figure 4. Zone of inhibition of *Aspergillus* fumigates against *Staphylococcus aureus* , *Streptococcus pyogenes* and *Pseudomonas aeroginosa*.

From the above Table 1. It shows that only *Aspergillus fumigatus* showing degree of bacterial inhibition to the tested organisms (*Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeroginosa*) which indicate that only *Aspergillus fumigatus* have pharmacological activities. Other fungi like *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* and *Penicillium* do not have pharmacological activities that is why they did not give any degree of bacterial inhibition against tested organisms like *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas* aeruginosa.

Table 1. Antibacterial Activity of fungal metabolites against pathogenic bacteria

Fungal extract	Zone of inhibition (mm)		
	Pseudomonas aeroginosa	*Streptococcus pyogenes*	*Staphylococcus aureus*
Aspergillus fumigatus	33 mm	27 mm	37 mm
Aspergillus flavus	_ _	_ _	_ _
Aspergillus niger	_ _	_ _	_ _
Rhizopus	_ _	_ _	_ _
Penicillium	_ _	_ _	_ _

Conclusion

According to the study it was concluded that *Aspergillus fumigatus* (that isolated from spoiled food) was have capability to inhibit clinical pathogenic bacteria like *Pseudomonas aeroginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* while the other like *Aspergillus niger* , *Aspergillus flavus* , *Rhizopus* and *Penicillium* did not show any antibacterial activity , was checked by using Kirby Bauer method. These result indicate that *Aspergillus fumigatus* extract which isolated from spoiled food showed a wide range of anti bacterial activity and have potential to inhibit the growth of pathogenic bacteria. .Thus *Aspergillus fumigatus* extract can be use as antibiotic. The study is designed that whether fungi can inhibit the growth of pathogenic micro-organisms and this work also demonstrate an interesting bottom-up approach to the discovery of new antimicrobial compounds.

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